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THE UNIVERSITY OF ALBERTA

Yield, sensory properties and nutritive qualities of quarg produced from lactose hydrolysed and high heated milk

by

© Hasmukh B. Sheth

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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OF MASTER OF SCIENCE
IN
FOOD PROCESSING

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA
Fall, 1988

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(SIGNED)---H. B. Sheth---

PERMANENT ADDRESS:
P.O : TENTALAV
Dabhoi, Baroda
Gujarat, India

DATED--19/7/-1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies and Research for acceptance, a thesis entitled **Yield, sensory properties and nutritive qualities of quarg produced from lactose hydrolysed and high heated milk** submitted by **Hasmukh B. Sheth** in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in FOOD PROCESSING.

Supervisor

Date July 18/83

ABSTRACT

Quarg was produced from homogenized milk with or without lactose hydrolysis or prior heating to 95° C for 15 min. Increased yields of 9.2 % and 13.3 % were obtained for unhydrolysed and hydrolysed high heated milk, respectively, in comparison to the corresponding unheated milks. The recovery of whey proteins in high heated milks was 40.5 % in unhydrolysed milk and 42.7 % in hydrolysed milk. No effect ($P \geq 0.05$) of lactose hydrolysis on yield was observed. The time required to attain the desired pH 4.5 in quarg fermentation was reduced in lactose hydrolysed milk by 7.1 % and 4.6 % for the unheated and high heated milk, respectively. The results of sensory evaluation indicated that the heating process resulted in graininess while the hydrolysis alone or combined with high heating induced an unclean flavor in the quarg. In nutritional evaluation by bio-assay methods, no differences ($P \geq 0.05$) were found in protein digestibility among any of the quargs studied, however, PER, BV and NPU values indicated that lactose hydrolysis combined with high heating of milk had a negative effect ($P \leq 0.05$) on the nutritional value. Amino acid analysis of quarg samples indicated possible lowering of lysine content for the hydrolysed quargs.

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1. INTRODUCTION

Quarg can be described as an acid coagulated, flocculated casein obtained by lactic acid fermentation (Siggelkow, 1984). The ideal characteristics of quarg are milky white color, homogeneously soft and mildly supple or elastic body, no appearance of water or whey, no dryness or graininess and clean, mildly acidic flavor. Quarg, classified as a soft cheese (Friis, 1981) is generally produced from skimmed milk, although it is available in the 0 to 40 % fat range (Lang, 1980). Table 1.1 shows the typical composition of the quargs made from whole and skimmed milk (Sohal, 1986). In Germany, France and Belgium quarg is consumed plain or in combination with fruits, jellies, whipped cream, vanilla or fruit flavors. It is also used on baked potatoes, in salads and bakery products (Claydon *et al.* 1972). West Germany is one of the highest quarg consuming countries (Jelen, personal communication) ; the popularity of the product in this country is shown in Table 1.2. The per capita consumption of quarg is 6.4 kg in West Germany (Siggelkow, 1984), 3.5 kg in France and 2.0 kg in Belgium and Luxembourg (Mann, 1978). Quarg is consumed in some form by 90 % of West Germans (Lang, 1980) with 15 % consuming at breakfast (Kroger, 1980). The minimum requirement for dry matter in quarg is 16 % in Denmark while in West Germany it is 17.5 % (Friis, 1981).

In North America including Canada, quarg is slowly gaining acceptance. In Canada quarg is classified as a specialty cheese and most of the specialty cheeses are imported from the Europe and the U.S.A. (Sohal, 1986). According to Agriculture Canada statistics, (1984), the per capita consumption of specialty cheeses has increased from 1.39 kg to 4.09 kg in last ten years.

A product similar to flavored quarg named 'Shrikhand' is very popular in India mainly in the states of Gujarat, Maharasthra and Karnataka. Shrikhand is made by lactic coagulation of milk and expulsion of whey from the curd followed by mixing of sugar, flavoring and

Table 1.1 Composition of quarg produced from whole and skim milk.

Components	Skimmilk	Whole milk
	(%)	(%)
Total solds	17.5	27.0
Protein	12.1	9.9
Butterfat	Traces	13.4

(Adapted from Sohal, 1986)

Table 1.2 Growth of dairy products in the Federal Republic of Germany (in tonnes).

Year	1950	1960	1970	1980	1982
Cheese (Total)	220,421	349,376	564,238	894,741	962,577
Hard/semisoft cheese	115,225	137,218	193,058	380,271	418,945
Quarg(fresh cheese)	47,066	125,362	266,753	367,695	393,037
Sour milk and cooked cheese	21,307	26,325	29,213	27,284	27,094
Processed cheeses	36,823	60,471	75,214	119,491	123,501
Whey cheese	25,408	38,198	23,837	16,412	17,070

(Adapted from Siggelkow, 1984)

spices. Shrikhand is served as a summer dessert and is in high demand especially in the March to June period. The average composition of this product is 34-40 % moisture, 43-45 % sugar, 4-6 % fat and 10-12 % solids-not-fat (Personal experience, Sugam Dairy,India ; Patel and Abd El Salam, 1986).

In recent years, several heating processes like Centri whey, Lactal and Westfalia, which recover whey proteins in quarg, have found industrial applications. Also, many workers have expended considerable effort to study the effect of lactose hydrolysis on the economics of quarg production. It seems that there is very little information on the influence of lactose hydrolysis alone or in combination with high heating on the sensory qualities, fermentation time , yield and the nutritive quality of resulting quarg.

This study was undertaken to compare the yield, the fermentation time, the sensory characteristics, the nutritive value and the amino acid profile of quarg samples produced from homogenized pasteurized milk, high heated (95^{o} C for 15 min) milk, 85 % lactose hydrolysed milk and lactose hydrolysed, high heated milk. Our objective was to evaluate the alleged benefits of these processes in terms of increased yield, process improvements and nutritional properties of the product in contrast to possible detrimental effects on product quality.

2. REVIEW OF LITERATURE

2.1 INDUSTRIAL PROCESSES OF QUARG MANUFACTURE

2.1.1 Introduction

Quarg is an acid coagulated, flocculated casein with a high moisture content, usually in the 70-80 % range. It is produced from milk by lactic acid fermentation sometimes accompanied by addition of rennet and finally separation of the whey. Until the late 1950's, traditional quarg manufacture involved the use of muslin bags for the separation of whey. At present, many plants in Europe follow modern methods of quarg manufacture, which are highly automated and controlled. These methods include separation of whey from the coagulum by a quarg separator, the first of which became operational in 1961 (Kroger, 1980); cleaning in place; moisture control; and yield optimization.

2.1.2 Traditional quarg production

Figure 2.1 shows the flow diagram of the traditional quarg production as described by Gungerich (1981). Good quality skim milk is inoculated with a buttermilk culture. Approximately one hour after the addition of the starter culture, the pH of milk drops to 6.5-6.4 and at this point rennet solution is added to the milk. Typical incubation temperatures range from 22-30^{o} C, but the lower incubation temperatures are preferred for the control of bacterial count (Sohal, 1986). When a pH of 4.5 is reached, curd is cut and whey is separated by cheese cloths lined in a perforated basket or in muslin bags. Whey is allowed to drain for a period of 10 -12 hours after which the quarg is thoroughly mixed and stored at the refrigerated temperatures. Gungerich (1981), Friis (1981) and Sohal (1986) have reviewed this process in detail.

2 1.3 Thermo processes

Quarg produced by traditional method has a limited shelf life. Also, traditional processes

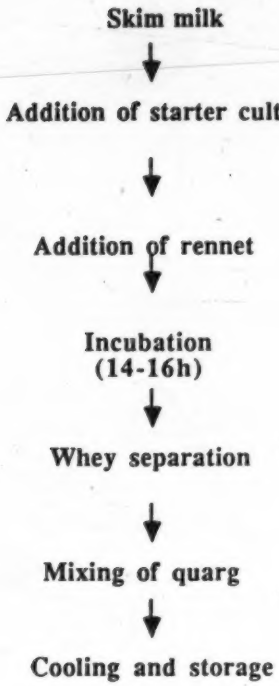

Figure-2.1 Traditional process of quarg production (Adapted from Gungerich, 1981).

have several limitations such as difficulties in moisture control in the final product and the quantity of quarg produced per day. In recent years a rise in the price of milk made it necessarry to look for processes that can increase the yield of quarg. Different processes of high heating of milk/whey for quarg production are schematically shown in Figure 2.2. These processes help retain significant amount of whey proteins in the product by their denaturation. According to Lang (1980), the economy of quarg manufacture can be determined by the degree of whey protein retention in the product. Dolle (1978) has described Lactal and Centri whey processes, while Siggelkow (1984) has reviewed the Westfalia process of quarg production.

In the Lactal method, quarg is produced by the normal procedure. A protein precipitate is obtained by heating the resulting whey to 95^{o} C, heated whey is cooled, and concentrated in a nozzle type separator to a dry matter content required for quarg. The protein recovered in this way is mixed with the regular quarg . Whey obtained from the normal process is also heated to 95^{o} C to precipitate protein in the Centri-whey method. The cooled whey is separated by a self-cleaning separator, which ejects slurry containing the precipitated proteins. This slurry containing whey protein precipitate of 12-14 % total solids is pasteurized, cooled and carried back to the vat milk for next day's quarg production.

The Westfalia thermo process involves heating of skim milk to 85-95^{o} C for a few min. The skimmed milk is then cooled to the normal incubation temperature and quarg is produced . Sohal (1986), Lang (1980), Friis (1981), Gungerich (1981), Kroger (1980) and Mann (1978) have reviewed this process in detail. According to Siggelkow (1984), the Westfalia thermo process can recover 50 % of the whey proteins and can increase the quarg yield by 10 %.

2.1.4 Ultrafiltration process for quarg manufacture

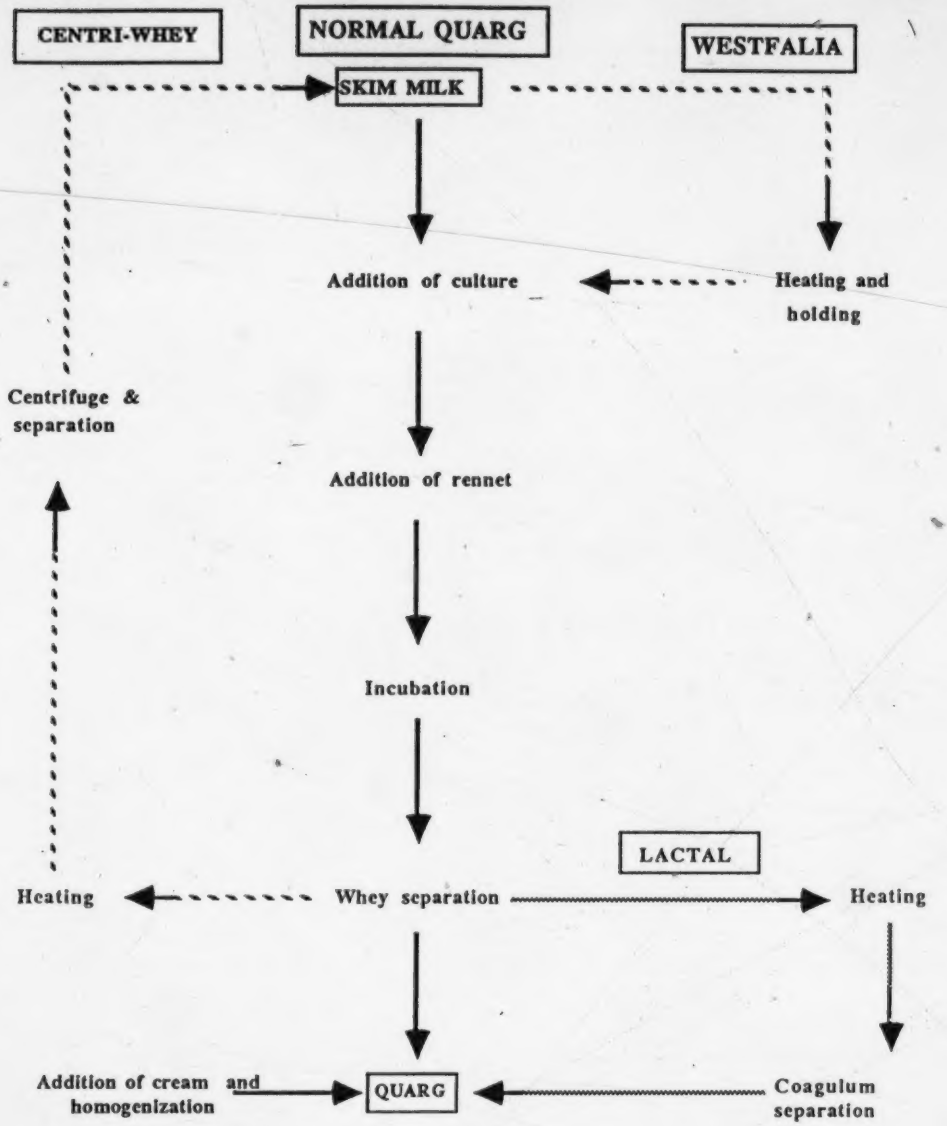

Figure - 2.2 Modern high heating processes of quarg production
(Adapted from Friis, 1981)

The ultrafiltration process has the ability to retain all the milk whey proteins in the product . Not only does this process increase the yield of the product but it also improves the nutritive value of the quarg as whey proteins have higher nutritional quality (Puhan and Gallmann, 1981). Friis (1981) has described quarg production by direct ultrafiltration of milk. However, quarg obtained by this method often develops a bitter taste during storage. This taste defect is associated with the higher amount of calcium content (3-times as high) than in traditionally manufactured quarg (Friis, 1981). This problem of bitterness can be overcome by preacidification of milk immediately before ultrafiltration. Acidification converts micelle bound calcium into dissolved form which can pass through the membrane with permeate.

2.2 LACTOSE HYDROLYSIS

2.2.1 Lactose

Lactose is a disaccharide made up of the monosaccharides, glucose and galactose. Cow's milk contains on the average 4.8 % lactose, although it is found in the 2.0 to 7.0 % range in different species of other mammals. Lactose is less sweet than fructose and glucose. For example, 1.8 % glucose or 0.8 % fructose solutions are as sweet as 3.5 % solution of lactose . Also, lactose is sparingly soluble in water when it is compared to the glucose. Nickerson (1974) has reviewed the properties of lactose in detail.

2.2.2 Mechanism of lactose hydrolysis

The enzyme lactase also known as β–galactosidase (E.C.3.2.1.23) acts specifically on lactose. This enzyme breaks the β–1-4 glycosidic linkage and liberates the monosaccharide sugars, glucose and galactose. The active site of this enzyme has one -SH and one imidazole group. The splitting of lactose is characterized by bimolecular substitution and nucleophilic (SN2) like displacement. In this reaction a sulfhydryl group at the active site acts as an acid

to protonate galactosidic oxygen atom, while the imidazole group acts as an electron pair donating reagent (i.e. nucleophile), which attacks the nucleophilic center at the anomeric carbon of the galactose. In the galactosyl removal, the sulfhydryl anion acts as a base to abstract a proton from water, which assists in the attack of OH^- at the anomeric position (Shukla, 1975).

It is well known that the β−galactosidase also catalyzes the formation of oligosaccharides. This enzyme effects the transfer of the galactose moiety to the acceptor molecule such as a monosaccharide or a polysaccharide by the process called 'transgalactosidation'. The source of enzyme, substrate concentration, pH, temperature and the nature of substrate influence the number and types of oligosaccharides formed. Shukla (1975) has reviewed the theory of lactose hydrolysis in detail.

2.2.3 Different methods of lactose hydrolysis

Originally, lactose hydrolysis in milk was studied in conjunction with the lactose intolerance problem. Alhough this problem was recognised in 1950's it was not possible to prepare low-lactose dairy products until the development of a commercial enzyme preparation from the microbial sources. In recent years, an attempt has been made to apply lactose hydrolysis to the production of dairy products in order to gain various technological advantages such as reduction in fermentation time, increased yield and a product with a sweeter taste. For the hydrolysis of lactose either soluble or immobilized lactases have been used.

In a possible batch process scheme for industrial lactose hydrolysis, milk is pasteurized and cooled to the incubation temperature. The enzyme lactase is then added to the milk and left for a fixed time. When the desired level of lactose hydrolysis is achieved, milk is re-pasteurized to terminate the reaction. However, by this process the enzyme is lost with the milk. In an attempt to improve the economy of the lactose hydrolysis, several processes

including ultrafiltration have been used to recover and/or reuse the enzyme. Nijpels (1977) has described the industrial process scheme for the hydrolysis of lactose in milk, whey or UF permeate with recovery of the enzyme by ultrafiltration. Huffman-Reichenbach and Harper (1982), Sheth *et al.* (1987) among others have also studied the recovery of enzyme by the ultrafiltration.

Immobilization of the β-galactosidase is another way allowing for reuse of the enzyme. Nijpels (1977) has discussed reactor systems in which enzyme is immobilized either by chemical or physical binding to an organic or inorganic support or by entrapment in fibers. According to this review, cellulose triacetate can be used for the entrapment while porous silica beads may be used for binding enzyme by cross-linking with glutaraldehyde. Valio dairy in Finland uses an immobilized enzyme system, using *A. niger* lactase to hydrolyze 20,000 L of whey per 16-20 h at 25-30^{o} C (Nijpels, 1977 ; Jelen, personal communication).

2.2.4 Technological reasons for lactose hydrolysis and its applications in the manufacture of dairy products

The sweetness of the lactose hydrolysed milk can be advantageous in the production of dairy products like flavored quarg, or shrikhand which require addition of sugar. Lactose hydrolysed milk has been used for the manufacture of quarg and cottage cheese; the wheys produced contained 55-60 % lactose in the hydrolysed form (Nijpels, 1981). Use of lactose hydrolysed whey obtained by this way could have an additional economic and functional value in dairy products like ice-cream and whey based beverages.

Also, because of hydrolysis, sparingly soluble lactose is transformed into more soluble glucose and galactose. Therefore, lactose hydrolysed milk is suitable for the production of concentrated dairy products, where lactose has a tendency to crystallize. Lactose hydrolysis to the extent of 25-30 % can prevent crystallization in sweetened condensed milk or in a

whey-based spread (Patocka and Jelen, 1988). Nijpels (1981), has reported that lactose hydrolysis improved storage stability and taste of frozen concentrated milk products.

Another technological advantage of lactose hydrolysis can be the reduction of setting time in the production of fermented dairy products. Thompson and Gyuricsek (1974) found that lactose hydrolysed yoghurt had a faster rate of acid production and decreased setting time. O'Leary and Woychick (1976) have compared some of the chemical properties of yoghurts made from the control and lactase treated milks. According to these authors, yoghurt made from lactose hydrolysed milk required less time to attain a pH of 4.5, contained more lactic acid and received a significantly higher scores for the flavor than the yoghurt obtained from the unhydrolysed milk . However, in a similar series of experiments Ismail *et al.* (1983) found that the yoghurt made from the lactose hydrolysed milk received lower scores for flavor, body and consistency. Nijpels (1981) reported that lactose hydrolysis reduced the set time in the fresh cheeses like cottage cheese and quarg, produced firmer curd and reduced losses due to cheese fines. According to this report, lactose hydrolysis in milk can increase yield up to 10 %.

Thompson and Brower (1974) noted in their experiment that, in cheese obtained from lactose hydrolysed milk, ripening period was reduced by 25-33 % , the coagulum was firmer and the curd tended to mat and hence more agitation was necessary. There was a considerable reduction in the cooking time, and shortening of cheddaring time by 40 min from usual 2-2.25 h. Cheddar cheese produced from lactase treated milk developed the same flavor in half the time required for the control cheese.

Weaver *et al.* (1978) have reported data on proteolysis and development of amino acid patterns during the ripening of cheddar cheese produced from the lactose hydrolysed milk. Marschke and Dulley (1978) claimed that 20 % lactose hydrolysis is sufficient to obtain

decrease in the ripening time. Accelerated cheese ripening in cheddar cheese by the addition of β–galactosidase has been reported by Cardwell and Prombutara (1976), Gooda *et al.* (1981), Thompson and Brower (1974 and 1976) and Ridha *et al.* (1983, 1984). Cheese obtained from the lactose hydrolysed milk was sweeter in taste compared to a control.

In addition to these technological advantages, lactose hydrolysed milk is very useful for the lactose intolerant consumers of dairy products.

2.2.5 Lactose intolerance

Lactose is found in the milk of all mammals. Other foods replace milk during the weaning period of the newborn and this, as a consequence, results in a gradual loss of intestinal lactase activity.Lactose intolerance in humans has generated a great deal of research interest and several studies have shown that a significant proportion of world's population is unable to digest lactose because of the deficiency of lactase in the intestinal mucosa. Lactose intolerant people are unable to absorb lactose, which then passes on and serves as a carbohydrate source for the intestinal microflora in the large intestine. Lactic acid, carbon dioxide and hydrogen are formed as a result of microbial action which, along with water drawn into colon, result in symptoms such as diarrhoea, flatulence, cramps and bloating (Hourigan,1984).The accelerated transport of food through the intestine, as a result of these symptoms, may cause loss of valuable proteins and minerals. The studies of Lisker and Aguilar (1978) and Payne-Bose *et al.* (1977) provide very useful information on the clinical implications of lactose intolerance.

2.3 HEATING OF MILK AND ITS EFFECTS ON CHEESE MANUFACTURE

2.3.1 Theoretical aspects

Normally, cow's milk contains about 33 g of proteins/L of milk. These proteins can be classified in two classes, namely, caseins and serum proteins. Of the total protein present in milk, casein part constitutes about 80 %. The casein fraction of the milk protein is remarkably stable to processing e.g. heat. The rigid structure of the casein micelle is so resistant to heat effects that it does not aggregate for appreciable time even at temperatures above 100° C ; its structure also resists breakdown during processing coditions such as homogenization (Dalgleish, 1981). However, when milk is subjected to a heat treatment, whey proteins are denatured and tend to associate with one another and with casein (Smits and Van Brouwershaven, 1980). The disulfide bridging, calcium linkages, hydrophobic bonding and hydrogen bonds are responsible for these associations (Zall *et al.*, 1983).

Heating is an important step involved in the processing of foods. Because of the heat treatment, some of the whey proteins are denatured; de Wit (1981), has defined protein denaturation as unfolding of the compact globular molecule into a less organized structure. Harland and Ashworth (1945) showed that heating of milk at 80° C for 45 min would denature 93-95 % of the whey proteins. The thermal stability of whey proteins during the heat processing has been evaluated by Hillier and Lyster (1979) and Fox (1981). Formation of disulfide linkages take place between the denatured β–lactoglobulin and the sulphydryl groups of κ–casein (Mckenzie, 1971 ; Sawyer *et al.*, 1963 ; Sawyer, 1969) and possibly also with α–S2 casein (Smits and Van Brouwershaven, 1980). At temperatures higher than 100° C, serum proteins are excessively bound to the casein micelle (Dalgleish, 1981). Patocka *et al.* (1986) have studied the effects of heat treatment on the solubility of whey proteins in cottage cheese whey in highly acidic conditions. Bernal and Jelen (1984) have shown the importance of Ca^{++} for stabilization of α–lactalbumin against heat denaturation at pH 4.5 or higher.

2.3.2 Suitability of high heated milk for cheese manufacture

High heating of milk incorporates whey proteins into cheese (Marshall, 1986). This is a very desirable effect, as it decreases the quantity of milk consumption for the production of 1 kg of cheese compared to that obtained from the unheated milk. Modler (1974) reported that high heated milk had a tendency to yield a cheddar cheese with excessive moisture and flavor and texture defects. Marshall (1986) has suggested that high heated milk is suitable for the production of Cheshire cheese which has a higher moisture content and more crumbly texture than cheddar cheese.

Wilson and Wheelock (1972) found that heating of milk inhibits the primary phase of rennin action due to complex formation between the κ–casein and β–lactoglobulin and a change in the distribution of Ca^{++}. Another explanation given by Morrissey (1969) for the increased clotting time was the deposition of calcium-phosphate complex formed during heating on the already formed caseinate- β–lactoglobulin complex.

The coagulation of milk by the enzyme rennin occurs by a two stage process. In the first stage, the κ–casein which stabilizes the casein micelles is hydrolysed (Wake, 1959) and then in the second stage the rennin altered micelles are precipitated in the presence of Ca ions. To prove the association of β–lactoglobulin with the κ–casein, Long *et al.* (1963) and Sawyer (1969) heated the isolated β–lactoglobulin and κ–casein together. The formation of β–lactoglobulin-κ–casein complex causes a considerable increase in the rennin coagulation time (Zittle *et al.*, 1962). Kannan and Jenness (1961) claimed that rennin action is inhibited by the heating of milk and this inhibition is mostly governed by the presence of β–lactoglobulin. Milk which has been high heated did not form a clot after rennin addition and therefore Hindle and Wheelock (1970) postulated that heating step completely inhibited the primary phase of the rennin action. However, when they added Ca^{++} ions to the milk, a clot was formed. This implied that the presence of Ca^{++} ions allowed primary phase to proceed normally.

2.3.3 Effects of high heating of milk on yield and sensory properties of cheese.

If cheese is produced from high heated milk, the yield of the resulting product is increased. Marshall (1986), examined the effect of milk heating (97^{o} C/15 sec) on the sensory properties of the cheshire cheese. He found that milk had a slight cooked flavor but this effect was not carried over into the cheese. Cheddar cheese produced from the high heated milk formed a soft coagulum. It was believed that this problem could be solved by adjusting the pH of milk meant for cheese production to 6.3-6.4 with lactic acid. Even after this adjustment, Marshall (1986) found that during cheddaring the curd blocks tended to fall apart and texture was poorly developed. Marshall further observed that the finished cheese was too moist, crumbly and had a lactic flavor. These were the typical characteristics of a cheshire cheese, so he concluded that heated milk was more suitable for that kind of cheese. The yield of the product (on a dry matter basis) was increased by heat treatment of the milk to the extent of 4.5 %.

Heating process has been also applied in the production of soft cheeses. If a high heating process is followed, the additional cottage cheese yield could be worth more than 40 million dollars per year and extra cheddar cheese made could be priced at more than 200 million dollars annually (Zall *et al.* ; 1983). White and Ray (1977) in their study found that high heating increased the yield but it impaired the textural quality of cottage cheese. Zall *et al.* (1983) have studied the effect of mild heating of milk (74^{o} C for 10 sec) on the yield of different varieties of cheese. They found that the increase was 5 % in cottage cheese, 4 % in cheddar cheese and 2 % in quarg cheese. They claimed that from heated milk, a good quality cottage cheese and quarg can be made. According to Siggelkow (1984), the Westfalia thermoprocess increased the quarg yield by 10 % by recovering 50 % of the whey proteins.

2.4 EVALUATION OF NUTRITIONAL QUALITY OF FOOD

2.4.1 Nutritional evaluation of protein quality

Biological methods of protein evaluation are methods based on the response of experimental animals to the protein under consideration. Biological methods of protein quality determination include protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and protein digestibility. Two terms, 'apparent' and 'true', are associated with the digestibility. The 'apparent' digestibility is defined as the ratio of the nitrogen absorbed by an animal to the quantity of nitrogen in food. 'True' digestibility of the protein is obtained by taking into account metaboloc faecal nitrogen (MFN), which is excretion of nitrogen from the biological system of an animal even if it is not fed protein.

The shortcoming of the digestibility method has been overcome by protein evaluation methods which are based on the growth of experimental animals under consideration e.g. PER, NPU and B.V.

The PER is defined as the weight gain per unit weight of protein consumed. Biological value is calculated as the ratio of the reatained nitrogen to the quantity of absorbed nitrogen. It is a direct measure of the protein which can be utilized in the synthesis of body tissues. The product of the digestibility and the B.V. is termed as the Net Protein Utilization (N.P.U.) which determines the usefulness of the protein.

Recently, the reliability of biological methods has been questioned (Bender, 1982). It has been suggested that if reliable amino acid data are available, the crude bio-assay methods are not required. However, reliable amino acid data are not available in many cases because amino acids like tryptophan during the preparation of the sample are lost and hence there is a need for bio-assay results to establish the meaning of the other simpler methods such as microbiological assays and dye-binding methods. Therefore there is no real substitute for a

biological assay. According to Harper *et al.* (1972), there is no protein source being accepted as a food without its being tested by a standardized rat-assay. For example the PER method is being used for the nutritional labeling of food in the USA. When a new method of assessing protein quality is proposed, it is always compared with the PER (Landers, 1975 ; Evanco *et al.*, 1977). However, PER and other biological methods are only based on the limiting amino acids and do not take into account other amino acids. Also PER varies with the food intake and the weight gain is assumed to be protein tissue. It is claimed that the estimation of the NPU is a very practical measure of protein quality. It takes into account both biological value and the digestibility.

Amino acid analyses of food proteins give some indication of the nutritional quality of the food. The chemical score can be calculated by comparing the amino acid which is lowest in quantity compared to the same amino acid in a reference protein (Block and Mitchell, 1946). However, the amino acid analysis has certain limitations namely : difficulty in distinguishing between protein-bound amino acids and free amino acids, destruction of amino acids such as tryptophan during hydrolysis before chromatographic estimation and also formation of unavailable components, for example with lysine. Also, validity of the chemical score is determined by the reliability of the reference protein.

From the study of all these methods, it seems that no method is perfect. Each method has its advantages and disadvantages and hence, depending upon specific requirement of the nutritional quality the appropriate choice of the evaluation method can be made.

2.4.2 Nutritional importance of specific milk proteins

Cow's milk protein contains 78-80 % casein in contrast to 20-30 % in human milk. Caseins are unique in their high content of ester-bound phosphate, a high proline content and low content of sulphur amino acids, especially cystine. Hambraeus (1984) discussed the

nutritional importance of casein and whey proteins in detail. According to this report, caseins are characterized by their micelle structure which contains calcium and inorganic phosphate. Thus caseins are known to represent some of the naturally occuring phosphoproteins. Besides their role as a source of amino acids, they also supply calcium and inorganic phosphate. Caseins play a very important role in the phosphorus balance in adults as well in newborn infants. In many countries, the dairy products constitute an important source of calcium in the diet. In Sweden, 75 % of the total dietary calcium is supplied from the consumption of dairy products.

From table 2.1 it is evident that whey proteins have low quantity of proline and arginine, but high amount of isoleucine, leucine, lysine and threonine, when they are compared to casein . As the latter four amino acids are the essential amino acids, this shows the nutritional superiority of the whey proteins over the caseins in cow's milk.

Though the exact role of β–lactoglobulin is not fully understood, it was suggested by Hambraeus (1984), that it plays a role in the phosphrus metabolism of the mammary gland. The other whey protein, α–lactalbumin, plays a key role in the regulation of the synthesis of lactose. Forsum (1974), claimed that the biological value of the α–lactalbumin is very high due to the optimum composition of amino acids for the human requirements. Milk also contains two iron binding proteins namely, transferrin and lactoferrin. The protein transferrin is known to play a very important role in the absorption of iron and also as an iron carrier in the body (Brock, 1980). According to Bullen *et al.* (1972), lactoferrin is responsible for the prevention of intestinal infections caused by *E. coli.*

2.4.3 Protein supplementation

The nutritive value of a protein alone may be markedly different than when estimated as a mixture of two or more proteins. The deficient pattern of amino acids of the various proteins

Table 2.1 Amino acids in casein, whey proteins and some otherfood proteins expressed as amino acid/total N (g/kg).

	Casein[a]	Wheyb	Beef[a]	Wheat[a]	Soya[a]
Isoleucine	345	476	301	204	284
Leucine	607	736	507	417	486
Lysine	518	704	556	179	388
Methionine	178	151	169	94	79
Cystine	23	174	80	159	83
Phenylalanine	334	224	275	282	309
Tyrosine	371	214	225	187	196
Threonine	297	527	287	183	241
Tryptophan	103	147	70	68	80
Valine	430	449	313	276	300
Arginine	239	175	395	288	452
Histidine	186	144	213	163	158
Alanine	196	341	365	226	266
Aspartic acid	455	766	562	308	731
Glutamic acid	1406	1231	955	1866	1169
Glycine	126	126	304	245	261
Proline	738	450	236	621	343
Serine	385	374	252	287	320

Sources : a Hambraeus (1984).
b Forsum *et al.* (1973).

can be corrected in whole or in part by mutual supplementation. In the process of supplementation, a low efficiency protein which has a poor amino acid balance is supplemented with another protein having an amino acids pattern, so that each tends to make up the deficiencies of the other. From this it is evident that the protein deficient in one or more amino acids is fortified by supplementation with another protein, rich in the essential amino acids which the first one lacked. This corrective process gives the final mixture with optimum level of amino acids to meet the requirements for growth and maintenance of an animal (Bressani *et al.*, 1972). McLaughlan and Morrison (1960) have shown the importance of supplementary relationships of proteins. In their experiment, rat diets with 10 % protein from bread which is deficient in lysine did poorly compared with rats fed bread diets supplemented with foods containing an excess of lysine such as cheese or fish protein concentrate.

Some of the whey proteins are transferred to quarg, cottage cheese and other similar dairy products if produced by high heat treatment of milk. According to Renner (1987), in quarg made from heated milk the total precipitated nitrogen increases from 77-79 % to 88-89 %. As discussed before, whey protein has high quantity of the essential amino acids compared to the casein. If a significant quantity of whey protein is transferred to the final product by heat treatment, it is likely to complement the deficiency of the essential amino acids of the caseins of the dairy product and hence increase the nutritive value.

2.4.4 Effects of processing on nutritive value of food

The amino acid composition of a food protein is very important in determining its nutritional quality, but other characteristics are also important especially in proteins that have been subjected to heat treatment during the manufacturing process. The susceptibility of protein to heat damage depends upon the protein source and it increases in the presence of various carbohydrates and other constituents of food. In the presence of reducing sugars,

some of the essential amino acids like lysine are lost through the well known browning reaction. Lactose hydrolysis of milk produces two reactive monomeric sugars, glucose and galactose and hence lactose hydrolysed milk has double the quantity of reducing sugars.

Because of the presence of more reactive sugar molecules, the rate of the browning reaction is faster in the lactose hydrolysed milk than the control.

As a result of the interactions between the functional groups, the formation of new linkages between the peptide chains take place. These linkages are resistant to hydrolysis by the digestive enzymes and hence impair the digestibility of the heat processed food. Bjarnason & Carpenter (1970) have proposed the several possibilities by which losses of amino nitrogen take place. It has been also shown by Bjarnason and Carpenter (1969) that proteins subjected to severe heating, the cross linkage formation between glutamine and lysine decreased the nutritive quality. They reported that heat damaged proteins are not accessible to the proteolytic enzymes.

Uptake of peptides across the mucosal cells is an important process in the protein digestion. Crampton *et al.* (1971) found that the unavailable peptides interfere with the uptake of intact peptides across the intestinal mucosal cells. One reason for interference of unavailable peptides in protein digestion can be the presence of these peptides in high concentration which saturate absoption sites involved in the transport of amino acids across the mucosal membrane.

Review of literature indicated that yield, process conditions, sensory properties and the nutritional quality are important factors for industrial quarg production. High heating processes have already found industrial applications in Europe. Quarg production from lactose hydrolysed milk has also been studied. However, very limited research has been carried out on the possible negative effects of these processes on the sensory and nutritional

properties of the resulting quargs. Also, very few research papers are available in English language, which makes it difficult to obtain detailed information on the effects of some of the modern quarg processing methods. It was therefore necessary to undertake this research in order to answer some of the questions related to sensory and nutritional aspects of quarg produced from high heated and/or lactose hydrolysed milk.

MATERIALS AND METHODS

3.1 INTRODUCTION

To meet the objectives of this project, the study was divided into 2 major sections:

1. A comparison of the yield, fermentation time, protein loss in whey and the sensory testing of quargs produced from homogenized pasteurized milk (Quarg A), high heated milk (95° C for 15 min, Quarg-B), 85 % Lactose hydrolysed milk (Quarg C) and lactose hydrolysed, high heated milk (Quarg D).

2. An evaluation of the nutritional quality of the four quarg products by bio-assay methods and the determination of their amino acid profiles.

3.2 MATERIALS

3.2.1 Milk

Homogenized pasteurized milk was supplied by the Lucerne Foods Ltd., Edmonton. The average butterfat of the milk was 3.5 % and the average dry matter content was 11.9 % as determined by the gravimetric method.

3.2.2 Rennet

Single strength pure calf rennet was obtained from Horran-Lally Co. Ltd., Mississauga, Ontario. The actual rennet activity, verified experimentally (Sohal, 1986), was 88 rennin units per mL.

3.2.3 Starter culture

Freeze dried Lactic culture (*Flora danica*) from Horran- Lally Co. Ltd., Mississauga , Ontario was supplied by the Neapolis dairy, a quarg producing plant in Didsbury, Alberta.

According to the supplier, the starter culture consisted of *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus diacetilactis* .

3.2.4 Enzyme for the lactose hydrolysis

The enzyme, ß-galactosidase, a liquid preparation from the yeast *Kluyveromyces fragilis* was supplied by Van Waters and Rogers Ltd., Lachine, Quebec.

3.2.5 Supplies for diets in the bioassay study

Animal Nutrition Research Council (ANRC) casein and vitamin mixture for the nutritional experiment was obtained from the Department of Animal Science, University of Alberta. A salt mixture necessary for the diet was prepared by mixing all the ingredients as suggested by the AOAC (1980) procedure for rat studies.

3.2.6 Chemicals

Necessary chemicals including sodium hydroxide, sulfuric acid, phenolphthalein indicator and petroleum ether used in chemical analyses were purchased from Fisher Scientific Co. (Fair Lawn, New Jersey 07410, USA.)

3.3 PRODUCTION OF EXPERIMENTAL QUARGS

3.3.1 Laboratory scale preparation of mother culture

Reconstituted skim milk with 10 % total solids was prepared from low heat skim milk powder. Milk was heat treated at 95° C for 30 min and cooled to 30° C before inoculating with the freeze dried lactic culture . After aseptically transferring the lactic culture, the milk was incubated at 30 $\pm$ .1° C for 8-9 hours until the pH dropped to 4.5. After the attainment of this pH, the mother culture was stored at 4° C.

3.3.2 Milk processing pretreatments for the production of quarg

Control quarg (Quarg A) was produced from homogenised, pasteurized milk . Quarg B was manufactured from the milk heated to 95° C and held at this temperature for 15 min in an oilbath and then cooled to 20° C by immersing in a chilled waterbath. The enzyme "Lactozym " was added to milk @ 2.3 mL per kilogram of milk before the manufacture of the Quarg C. Milk was stored at 5° C for 24 h to restrict the bacterial growth and as per manufacturer's instructions, the enzyme was then inactivated by heating the milk to 71° C for 16 seconds in a waterbath. The lactose hydrolysed milk produced as above was subsequently heated to 95° C and held for 15 min in an oilbath for the Quarg D.

3.3.3 Preliminary quarg production and sensory testing trial

To master the processing technique, a preliminary trial on quarg production was carried out with the help of a person experienced in the production of cheeses. Samples were drawn for the analyses of dry matter of quarg and percentage protein content of whey. Sensory testing was carried out with the help of 3 experts in the field of dairy products judging. Experts graded the samples by using the descriptive terms " not objectionable " , " slightly objectionable " and " very objectionable". The format used for this purpose is shown in Appendix 1.1.

3.3.4 Laboratory scale production of quarg

Control quarg was produced by following Sohal's (1986) method. Figure 3.1 shows the flow diagram of the above mentioned experimental method. Each batch of quarg was produced from 4 litres of milk. Approximately 10 litre size stainless steel containers were used for the production of quarg. All the containers were sterilized before use with live steam for 5 min In these containers the milk was warmed up to 30° C in a thermostatically

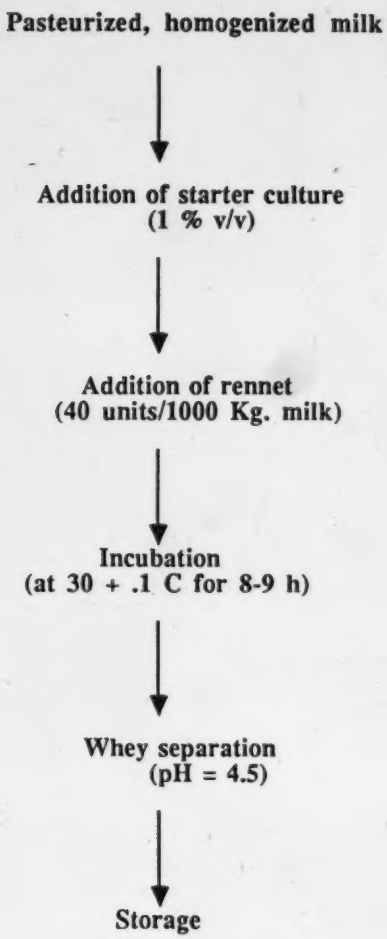

Fig. 3.1 Flow diagram of quarg production (Adapted from Sohal, 1986)

controlled waterbath (Precision Scientific Group, Chicago, Illinois, 60647). When the milk reached 30° C temperature, the starter culture was added at the rate of 1 % of the milk. The pH of each process was monitored using a digital pH meter. After 1 h, when the pH of the milk had dropped from 6.7 to 6.5-6.4, 1:10 dilution of rennet was added and the milk was incubated until a final pH of 4.5 was reached. Fermentation time for each process was determined , curd was broken with the help of a scoop and whey was drained for 10-12 h using several layers of cheese cloth. After the overnight drainage of whey, the quarg was thoroughly mixed and stored at 4° C temperature. Quarg and whey samples were drawn for analyses.

3.4 YIELD DETERMINATIONS

The quarg yield was calculated in two different ways : as a percentage of total solids recovered from milk, and as kilogram of quarg per kilogram of milk. Before starting the quarg production, the exact weight of milk used for all the processes was taken. The weight of quarg obtained from each process was determined at the time of packing. From the total solids analyses of the milk and quarg, the solids recovery into the product was calculated. For the calculation of the kilogram of quarg per kilogram of solids in milk, the quantity of the quarg was calculated from the solids recovered in the product and corrected for a product with 25 % total solids.

3.5 SENSORY PANEL TESTING OF QUARG

To confirm the results of the preliminary trial, sensory testing was carried out employing an 8 member panel. The panelists were students and staff of the Department of Food Science. All the judges were experienced in judging dairy products. Coded samples were evaluated for cooked and unclean flavor,grainy texture, sweetness and color by an adapted scoring method as described by Larmond (1977). For example for testing sweetness the terms " not sweet", "trace of sweetness", " slightly sweet ", " sweet", " very sweet " and " extremely sweet " were used to grade the sample. The anchor points used

ranged from 1 (for " not sweet ") to 6 (for extremely sweet). Before the taste panel experiments, the panelists were instructed about the typical characteristics of the product. However, they were not informed about the different treatments used. A reference sample (a duplicate of sample A without identification) was offered for the comparison of the experimental quargs. Appendix 1.2 shows the panel testing format.

3.6 NUTRITIONAL EVALUATION OF THE EXPERIMENTAL QUARGS

3.6.1 Production of freeze dried quarg

Quarg was produced 12 times following Sohal's (1986) method. This quarg was freeze dried, thoroughly mixed and vacuum packaged . Freeze dried quarg was used to prepare diets for the biological assays. Vacuum packaged quarg was stored at 4^{o} C until it was used for the preparation of the diet.

3.6.2 Preparation of diets

The proximate analyses of the quarg were carried out in triplicate. The required quantity for each component of the diet was calculated and all the ingredients were thoroughly blended in the mixer. The casein diet required in the nutritional experiment was prepared by using the ANRC casein. It was also necessary to maintain a group of rats on protein free diet in the bio-assay method of the protein evaluation. Corn starch was used as a base material for the protein free diet. Other ingredients were similar to the quarg diets and the same preparation procedure was followed.

3.6.3 Nutritional experiment setup

The nutritional study was carried out in the Department of Animal Science, University of Alberta. Environmental conditions were maintained as described in the literature (Miller

and Lachance, 1977). Rat (*Sprague dawley* species, obtained from Biosciences services, University of Alberta) was used as an experimental animal. The temperature of the animal room was maintained between 22-24° C. The relative humidity of the room was 40 %. Rats were housed in cages individually. There were in total, 11 groups, each containing 5 males and 5 females. On the first day of the assay the age of all the rats was 26 days. Feed dishes were large enough to allow true *ad libitum* feeding. Every other day water and the content of the feed dishes were changed.

3.6.4 Rat handling

The adaptation period for rats was 3 days. After receiving them from Biosciences, they were housed individually in metal cages and kept on a casein diet. According to literature, the mean weight difference of the test groups on the first day of the assay should not be large (Miller and Lachance, 1977). Appendices 1.3, 4 and 5 show the weights of the individual rats and mean weight of each group on the first day of the experiment. Chloroform was used to sacrifice rats after a specific time interval. The groups designated for the NPU and PER purpose were sacrificed after 10 and 28 days respectively. The weight of each rat of the PER groups was measured every seventh day at a fixed time.

3.7 CHEMICAL ANALYSES

The various chemical tests performed on the quarg included protein , dry matter and fat. Glucose and lactose content of the lactose hydrolysed milk was estimated. The moisture content of the sacrificed rats was also determined.

3.7.1 Moisture content

AOAC method # 16.233 (AOAC, 1980) was followed to determine the moisture content of the quarg. Triplicate samples of 1.5-2.0 g were placed in a forced air oven at 99-100° C for three hours. The cooled samples were weighed and percentage moisture was

determined by the weight difference .

3.7.2 Protein

Protein content of the freeze dried quarg was estimated by the Kjeldahl method (# 16.245; AOAC, 1980). The percentage protein content was obtained by multiplying the percent nitrogen with the factor of 6.38.

3.7.3 Fat

Fat content of the freeze dried quarg was estimated by adopting AOAC # 7.056 (1980) using the Goldfish extraction apparatus in the department of Animal Science, University of Alberta. Instead of anhydrous diethyl ether, petroleum ether (boiling range 35-60^{o} C) was used. This was the only change made from the above AOAC procedure.

3.7.4 Glucose

The concentration of glucose in the lactose hydrolysed milk was determined by the automatic YSI sugar analyzer model 27 (Yellow Springs, Ohio U.S.A).

3.7.5 Lactose

The lactose analysis of the lactose hydrolysed milk was provided by the Alberta Agriculture Food Laboratory, O.S. Longman Building. An HPLC method was followed using a carbohydrate column with a mobile phase, -H2O/CH3CN(1+3) - with refractive index detector held at 35^{o} C(Water Scientific Milford, Mass.)

3.7.6 Body water determination of the sacrificed NPU group rats

The rats meant for the Net Protein Utilization (NPU) determination were sacrificed at the end of tenth day using Chloroform . The weights of the dead animals were taken and

incision was made into skull, thoracic and body cavities as described by Miller and Bender (1955). Carcases were dried to a constant weight at 105° C for 48 h. After the drying period, the moisture contents of the rats were determined by the weight difference.

3.7.7 Estimation of the body nitrogen of the sacrificed NPU group rats

The nitrogen content of the sacrificed rats was calculated from their body water content. The following formula was found to correlate the body nitrogen and body moisture (Miller and Bender,1955):

$$Y = 2.92 + 0.02\,X$$

$$\text{Where, } Y = \frac{\text{Nitrogen (in g)}}{\text{Moisture (in g)}} \times 100$$

X = age in days

3.7.8 Calculation of the NPU

The NPU was calculated by applying Miller and Bender's (1955) equation as follows,

$$\%\ \text{NPU} = \frac{\text{Body N}^{*}\text{ of animal} - (\text{Body N of P.F.}^{**}\text{ group} - \text{N}^{*}\text{ intake of P.F.}^{**}\text{ group})}{\text{N}^{*}\text{ Intake of the animal}} \times 100$$

* N = Nitrogen , ** P.F. = Protein free

In the calculation of the NPU values, the average values of body nitrogen and nitrogen intake of protein free group were used. Appendices 2.10 and 2.11 show the raw data for these values.

3.7.9 Protein digestibility

The true digestibility was determined by the following formula (McDonald *et al.*, 1981) :

$$\% \text{ T. D.} = \frac{N^* \text{ intake} - (N^* \text{ in faeces} - \text{MFN})}{N^* \text{ intake}} \times 100$$

* = Nitrogen

The metabolic faecal nitrogen (MFN) was determined from the protein free group. The faecal matter of the protein free group was analyzed at the end of the experiment and the average value was taken as MFN. Appendix 1.6 shows the raw data for this calculation.

3.7.10 Derivation of the biological value

Biological Value was derived from the NPU and % T. D. as follows :

$$\% \text{ B.V} = \frac{\text{NPU}}{\text{T.D.}} \times 100$$ (Miller and Lachance, 1977)

3.7.11 Determinations of amino acid content of the freeze dried quargs

The amino acid analyses were carried out in the Amino Acid Laboratary of the Department of Animal Science, University of Alberta. Fat was extracted from the freeze dried samples of the quarg by the Goldfish extraction apparatus. The protein content and the dry matter were determined as before. After these analyses, the samples were refluxed with 6N HCl for 24 h. The hydrolysed samples were injected into Beckman 121 microcolumn amino acid analyzer. Table 3.1 shows the operating parameters of this equipment. From the peak area of the control and the sample, the quantity of each amino acid was calculated by comparison.

Cystine was determined as cysteic acid, while methionine was estimated as methionine

Table 3.1 Operating parameters of the 121 MB amino acid analyzer

Column size	2.8 X 300
Resin	AA-10
Resin bed height	200 mm
Buffer flow rate	10 mL per h
Ninhydrin flow rate	5 mL per h
First buffer	pH 3.28, o.20 N Na+
Second buffer	pH 3.90, 0.35 N Na+
Third buffer	pH 4.95, 1.40N Na+
Sample diluting buffer	pH 2.20, 0.20 N Na+
Regenerating reagent	NaOH, 0.20 N
Column temperaturen, high	65° C
Column temperature, low	50° C

sulfone in a separate set of experiments. For these determinations, all the quarg samples were oxidized with performic acid reagent for 4 h as described by Hirs (1963). The oxidized quarg was then hydrolysed with 6N HCl . The hydrolysed quarg was injected into the Beckman amino acid analyzer.

4. RESULTS AND DISCUSSION

4.1 PRELIMINARY TRIAL

One preliminary trial was carried out before repeating the whole process 4 times for a total of 4 batches per treatment. Quarg samples were analysed for the percentage total solids and the protein content of the whey resulting from the experimental quargs. The fermentation times of the quargs were determined when a desired pH of 4.5 was achieved. Quargs C and D were produced from 83 % lactose hydrolysed milk, while in subsequent experiments the extent of lactose hydrolysis for these quargs was 85 %. The preliminary sensory trial was carried out as described in the section 3.3.3 and the purpose of this study is clearly stated in that section. Table 4.1 summarizes the percentage total solids, percentage protein content of whey, the fermentation time and the sensory attributes including color acceptability, flavor evaluation and the texture characteristics of the experimental quargs. The sensory testing in this trial was based on experience of the experts and hence was a subjective evaluation.

From Table 4.1, it can be seen that higher amounts of total solids were recovered in quargs produced from the high heated milks. High heating process also decreased the quantity of protein lost in the whey. The hydrolysis of lactose in processes C and D slightly reduced the fermentation time.

In two replicates of the preliminary sensory trials, which involved three expert judges, quargs A,B and C were found to have acceptable color all six times. Slightly objectionable (4 times) and very objectionable (2 times) yellow discoloration was observed in quarg D. Product A was mildly acidic in flavor. Quargs obtained from the high heating process (i.e. B and D) were found to have a slightly objectionable flavor once and 6 times, respectively. Quarg C was judged as slightly unclean in flavor 3 times. Texture characteristics of the experimental quargs showed that product obtained from unheated milks (i.e A and C) were

Table 4.1 Results of the preliminary quarg production trial

Parameter	Quarg A	Quarg B	Quarg C	Quarg D
T.S. (%)*	59.20	66.45	58.60	64.60
Protein in whey (%)	0.80	0.44	0.78	0.44
F.T.** (h)	8.25	8.00	7.50	7.50
SENSORY ATTRIBUTES(No of observations)***				
Color (-)	6	6	6	0
(+)	0	0	0	4
(++)	0	0	0	2
Flavor (-)	6	5	3	0
(+)	0	1	3	6
(++)	0	0	0	0
B&T (-)	6	2	6	2
(+)	0	3	0	4
(++)	0	1	0	0

*total solids recovered in quarg

**Fermentation time

***(-)= Not objectionable, (+)= slightly objectionable
(++)= very objectionable.

B&T = Body and texture

smooth. Once very objectionable and 3 times slightly grainy texture was found in quarg B while quarg D was graded as slightly grainy 4 times.

4.2. COMPARISON OF YIELD IN EXPERIMENTAL QUARGS

In any commercial process yield is of prime importance because it effects the profitability of the product line. Table 4.2 describes the average values of percentage milk solids recovery in quarg and percentage protein content of whey of the 4 quarg replicates. Appendix 2.1 summarizes data for the percentage total solids recovery and percentage protein of whey from which the average values which are shown in Table 4.2 were calculated. From the yield (corrected for a product with 25 % total solids), the quantity of milk required to produce 100 kg of quarg was calculated. When the heat processing step was included in the production of quarg, the yield of the product from the process-B increased by 9.2 % compared to the process A while 13.3 % increase in yield was obtained in process D compared to the process C. No significant difference ($P \geq 0.05$) was found between the yields of quargs A&C and B&D. Similarly, protein content of quarg B whey (0.47 %) was reduced compared to the quarg A whey (0.79 %) and in quarg D whey (0.43 %) compared to the quarg C whey (0.75 %). Visually, high heated milks produced firmer curds and clearer wheys than unheated milks. The higher yield in the quargs produced from high heated milks was obviously due to the greater whey protein recovery in quarg. Also, because of firmer coagulum obtained in the high heated quargs, there may have been less loss due to the cheese fines. For the process B the percentage milk solids (67.25 %) was significantly different ($P \leq 0.05$) from the control quarg (61.56 %). Similarly, quarg D had a significantly ($P \leq 0.05$) higher milk solids recovery (64.95 %) than quarg C (57.33 %). Thus our results are in agreement with the resuts of Zall et al. (1983) regarding the increase in the yield of the product obtained from high heated milk. As in our case heating was more severe, the

Table 4.2 Milk solids recovery, fermentation times of the experimental quargs and the protein content of the wheys .

Process	Qty. of Milk required to produce 100 kg quarg with 25 % T.S. (kg)	Milk solids recovery in quarg (%)	Fermentation time (h)	Protein content of whey (%)
A	341.3	61.56 ± 1.19^{a}	8.18 ± 0.06^{a}	0.79 ± 0.03^{a}
B	312.4	67.25 ± 0.68^{b}	7.99 ± 0.06^{a}	0.47 ± 0.02^{b}
C	366.5	57.33 ± 1.95^{a}	7.60 ± 0.07^{b}	0.75 ± 0.03^{a}
D	323.4	64.95 ± 1.33^{b}	7.62 ± 0.09^{b}	0.43 ± 0.03^{b}

Data in each column followed by the same letter are not significantly ($P \geq 0.05$) different.

recovery of the total solids was higher compared to Zall's work. However, our results fail to confirm the claim of Nijpels (1981) regarding the increase in the yield of cottage cheese and quarg obtained from the lactose hydrolysed milk. This has been shown by the percentage protein content of the whey of the process C which is discussed in the next section.

The increase in yield of the process D can be attributed to the high heating process rather than lactose hydrolysis. Process D was found to be similar to the process B when the percentage total solids content and the protein content of both the processes were compared.

Table 4.2 also summarizes the average values of four replicates for the time required to attain a pH of 4.5. Appendix 2.1 lists the fermentation times of the experimental processes. The fermentation time was slightly reduced in the lactose hydrolysed milks. Quarg C required 7.1 % (35 min) less time than quarg A and for quarg D the time was 4.6 % (22 min) shorter than that for quarg B. No significant difference ($P \geq 0.05$) was found between the setting times of quargs A & B and C & D. The application of β–galactosidase gives rise to an increased rate of acid production . The possible explanations for the shorter time requirement to attain a pH of 4.5 for hydrolysed milk may include : i/ the elimination of steps in the substrate catabolism for the dairy bacteria, ii/ faster growth of bacterial culture resulting in more acid production and iii/ an alteration in the carbohydrate metabolism of the cells. By visual observation it appeared that the curd obtained from the lactose hydrolysed milk (i.e. process C) was not firmer compared to the control as reported by Nijpels (1981). Table 4.2 indicated that the protein content of wheys from the processes A and C was the same and thus it contradicts Nijpel's (1981) observation of saving solids as cheese fines if product is obtained from the lactose hydrolysed milk.

4.3 SENSORY EVALUATION OF EXPERIMENTAL QUARGS

To confirm the results of preliminary sensory testing by experts, panel testing was carried out at the end of the fourth quarg production replicate. Sensory testing was done as described in the section 3.5. Table 4.3 describes the results of the sensory evaluation of the experimental quargs. Appendices 2.2 to 2.5 summarize the data for the individual sensory parameter tested i.e. color, sweetness, unclean flavor and texture from which average values were calculated . Quarg A was of milky white color (average 1.0 point), while slight traces of off color were observed in quarg B (average 3.13 points) and quarg C (average 2.50 points). Quarg D was graded (average 4.13 points) to have definite yellow discoloration. This defect in quargs B, C and D can be related to heat treatment and browning reaction. The quarg C milk was heated to 71° C for 16 seconds for inactivation of the enzyme. This might have induced the browning reaction and as a result yellow discoloration. Almost no cooked flavor was noted in quargs A (average 1.25 points) and C (average 1.88 points) while in the case of quargs B (average 3.88 points) and D (average 4.25 points) the cooked flavor was definite. All the samples of quargs A and B were clean and mildly acidic in flavor while those from lactose hydrolysed milks were unclean. Products C and D received average 3.63 and 3.88 points for this defect respectively. There is no clear understanding of the unclean flavor origin in lactose hydrolysed quargs in this study, but we believe that the enzyme source might be one of the factors inducing this undesirable flavor. Lactose hydrolysed quargs were significantly ($P \leq 0.05$) sweeter (3.63 points for C and 3.75 points for D) than unhydrolysed products (1.0 point for the quargs A and B). Quargs produced from unheated milk were smooth in texture (average 1.0 point for A and C) while those produced from high heated milks were grainy (average 3.13 points for the quarg B and 3.38 points for the product-D).

It seems that the graininess in quags B and D could be alleviated by a proper homogenization treatment. 'Maska', a quarg like product used for the preparation of shrikhand, shows the same grainy texture after removal of whey from the coagulum

Table 4.3 Sensory characteristics of experimental quargs*

Quarg	Color	Cooked Flavor	Unclean Flavor	Sweetness	Grainy Texture
A	1.0 ± 0.0^{a}	1.25 ± 0.43^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
B	3.13 ± 0.60^{b}	3.88 ± 0.60^{b}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	3.13 ± 0.6^{b}
C	2.50 ± 0.86^{b}	1.88 ± 0.60^{a}	3.63 ± 0.48^{b}	3.63 ± 048^{b}	1.0 ± 0.0^{a}
D	4.13 ± 0.60^{c}	4.25 ± 0.66^{b}	3.88 ± 0.60^{b}	3.75 ± 0.66^{b}	3.38 ± 0.48^{b}

* 1 signifies no effect while 6 indicates pronounced effect.

Data in each column followed by the same letter are not significantly ($P \geq 0.05$) different.

(personal experience). When maska is mixed with cream, sugar and spices in a scraped surface heat exchanger and agitated for 30 min., the shrikhand obtained is silky smooth in texture.

4.4 NUTRITIONAL EVALUATION OF FREEZE DRIED QUARGS

Table 4.4 shows the average composition of the freeze dried experimental quargs. In nutritional studies, diets for rat feeding were prepared as per AOAC (1980) recommendations. Table 4.5 shows the composition of a suggested diet prepared by the AOAC (1980) method. Table 4.6 shows the proximate analyses of the diets used in this study. Fat contents of the experimental diets were higher compared to the AOAC diet because the freeze dried quargs from which diets were prepared were rich in fat (Table 4.4). However, other components like protein, salt, vitamin and fiber were similar to the ideal diet. As fat content in all the diets was similar, it should not have affected the results. For the estimation of body nitrogen (for the determination of the NPU) Miller and Bender's (1955) method was used. Although it is an indirect method i.e. it correlates body water content of animal to the body nitrogen, it was shown by these scientists that it is very precise , fast and easy compared to the other methods of body nitrogen determination like carcass method, which is tedious.

Table 4.7 summarizes the results of the bio-assay methods. Appendices 2.6 to 2.11 list the raw data for the different biological methods. There were no significant differences found in the protein digestibilities of any of the quargs studied, however, quarg D was found to have lower PER, B.V. and the NPU values when it was compared to quargs A, B and C ($P \leq 0.05$, Duncan's multiple range test).

The lower PER, B.V. and NPU values in quarg D likely can be attributed to the

Table 4.4 Proximate analyses of the freeze dried quargs (%)

Product	Protein	Fat	Total solids
Quarg A	34.35	42.08	97.01
Quarg B	37.21	40.68	96.50
Quarg C	34.67	42.21	94.73
Quarg D	36.47	39.03	94.54

Table 4.5 Suggested (AOAC, 1980) diet composition

Ingredient	Quantity (%)
Protein	10
Fat	8
Salt mixture	5
Vitamin mix	1
Fiber	1
Moisture	5
Sucrose or corn starch	To make 100

Table 4.6 Compositional analyses of the test diets (%)

Diet	Protein	Fat	Dry matter
Quarg A	10.36	12.68	88.93
Quarg B	10.07	12.64	88.88
Quarg C	9.93	12.70	89.26
Quarg D	10.49	12.33	89.26
Casein	10.42	12.36	90.69
Protein free	0.14	12.96	89.91

Table 4.7 Nutritional values of the experimental quargs based on the reference casein

Diet	Digestibility*	PER**	NPU***	BV
Quarg A	92.84 ± 1.17[a]	2.43 ± 0.13[a]	70.71 ± 4.83[a]	76.20 ± 5.59[a]
Quarg B	93.05 ± 0.83[a]	2.55 ± 0.13[a]	68.54 ± 5.33[a]	73.68 ± 6.10[a]
Quarg C	92.65 ± 0.65[a]	2.49 ± 0.14[a]	71.41 ± 4.07[a]	77.08 ± 4.50[a]
Quarg D	92.96 ± 1.01[a]	2.20 ± 0.23[b]	62.74 ± 4.70[b]	67.53 ± 5.68[b]
Casein (Uncorrected)	93.55 + 1.92[a]	3.14 ± 0.18	69.75 ± 1.80	74.86 + 1.56[a]

Data in each column followed by the same letter are not significantly (P≥0.05) different.

* Actual digestibility values were not corrected for the experimntal quargs

** Correction factor for the PER : 2.50/3.14 =0.796

*** Correction factor for the NPU : 70.00/69.75 = 1.0035

nutritional loss due to the browning reaction. Process D is such that it makes all the requirements of browning reaction possible. As a result of lactose hydrolysis, there is more availability of reactive sugars as one molecule of lactose yields one molecule of glucose and galactose . Glucose and galactose in the presence of heat and proteins play a significant role in the induction of browning reaction and may have caused the nutritional damage.

Renner (1987) has claimed that the high heating process improves the nutritive quality of the product however results in this study did not indicate any improvement of the nutritive value of the quarg obtained after the high heating of milk. As shown in Table 4.8 in the production 100 kg quarg, the recovery of protein in quarg B and D was 0.91 kg and 0.95 kg more respectively. When the total protein recovery in product B and D was compared to the control, it was found to be 8.9 % more and therefore it may not have any significant effect on the PER and the NPU.

4.5 AMINO ACID ANALYSES OF FREEZE DRIED QUARGS

Oser *et al.* (1951) have suggested that it would be more appropriate to base protein rating on the contribution it makes in respect to all the essential amino acids. The development of chromatography procedures for the analyses of amino acids in foods has been of tremendous value to nutrition research. However, Knipfel *et al.* (1971) have reported that variation associated with the analysis of amino acids on automated analyzers was due to sample manipulation rather than analysis.

To examine the effects of different processes, all the freeze dried experimental quargs were analyzed for their amino acid content. Table 4.9 shows the average amino acid content (g/16g N) of two replicates of all the quargs studied. There was no consistent effect of any

Table 4.8 Quantity of protein in whey obtained from the production of 100 kg quarg (kg) and protein in 100 kg quarg

Process	Qty. of whey obtained from the production of 100 Kg. quarg with 25 % T.S. (kg)*	Protein in whey resulting from 100 kg quarg (kg)*	Protein in 100 kg quarg with 25 % T.S. (kg)**	% of protein in quarg compared to control
A	241.3	1.91	8.85	100.0
B	212.4	0.99	9.64	108.9
C	266.5	2.00	9.15	103.4
D	223.4	0.96	9.64	108.9

*Calculated from Table 4.2

** Calculated from Table 4.4

Table 4.9 Amino acid content of the experimental quargs

Amino acid	g/ 16g Nitrogen			
	Quarg A	Quarg B	Quarg C	Quarg D
Arginine	3.700	3.593	3.686	3.727
Histidine	2.931	3.037	2.872	2.984
Isoleucine	5.181	4.911	4.988	5.010
Leucine	9.324	9.619	9.321	9.753
Lysine	7.911	8.108	7.575	7.635
Methionine	2.800	2.596	2.704	2.653
Phenylalanine	5.552	5.302	5.444	5.413
Threonine	3.792	3.939	3.862	3.854
Tyrosine	5.571	5.475	5.664	5.616
Valine	6.862	6.357	6.888	6.712
Alanine	3.388	3.423	3.472	3.512
Aspartic	6.404	6.811	6.672	6.911
Glutamic	18.360	18.475	18.400	18.800
Glycine	1.819	1.809	1.826	1.856
Proline	6.963	6.345	6.874	6.481
Serine	4.968	4.864	4.935	5.038
Cysteine	0.526	0.628	0.639	0.627

Recovery as protein (N X 6.38) 97.7%, 96.9%, 97.5% and 98.2% for the quarg A, B, C and D respectively.

The amino acid tryptophan is not included in this analysis.

of the processes studied. The lysine content appeared lower in quarg produced from processes C and D. The reduction of total lysine content in quarg D can be associated with the obvious browning reaction. The heating of milk for the inactivation of the enzyme in process C also might have induced some lysine loss due to browning reaction.

The results for quarg D corroborate the observations from the biological methods, while in product C the heating for the inactivation of the enzyme might have not been severe enough to cause a significant deterioration of the nutritive value to be detected in the rat assays.

It appears that while the high heating process used increased the product yield, it caused some impairment of the sensory and nutritive qualities of the product especially in combination with lactose hydrolysis. Thus, industrial processors before the use of these innovative techniques must carefully weigh their advantages and disadvantages.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY OF RESEARCH FINDINGS AND CONCLUSIONS

Investigation of four different processes of quarg production indicated that the high heating process had a significant effect on yield. Processes B and D required 29 litres and 18 litres less milk per 100 kg of quarg produced (the solids recovered into quarg corrected for a product with 25 % total solids). Though the inclusion of whey proteins per 100 kg. production of the product was only 0.91 kg and 0.95 kg more in quarg B and D respectively, compared to the control, the expected inclusion of water associated with whey protein also increased the yield.

Although the heating condition was severe, the quarg B containing only lactose, underwent browning to a lesser degree and hence the yellow discoloration was significantly less than in product D. Quarg C was not significantly different from B in yellow discoloration, though it was produced from hydrolysed milk (and therefore it had more reactive sugar molecules than in quarg B), because it was heated only to 71^0 C for 16 seconds for inactivation of the ß-galactosidase. It was reasonable to find that the quarg D milk which contained higher number of glucose and galactose molecules and was also subjected to higher heat treatment, produced significantly higher discoloration than quargs A, B and C because of the browning reaction. The other unwelcome effects of heating were cooked flavor and grainy texture (Table 4.3). The only beneficial effect obtained by the use of lactose hydrolysed milk was sweeter taste of the resulting quarg compared to the control. However, the introduction of the lactose hydrolysis step imparted an unclean flavor into quarg. The reason for this effect is not known. Further study would be desirable to pinpoint the factors which give rise to this defect.

It also seemed that the browning reaction played a significant role in lowering the PER, BV and the NPU of the quarg D compared to the control. Its effects on the lower lysine contents of quargs C and D was evident.

From the results obtained in this study, it can be concluded that, by adopting process B, the profitability of the quarg production can be increased. However, high heating of milk induced slight grainy texture and cooked flavor in quarg B. In these experiments, the fermentation time of the lactose hydrolysed quarg was reduced, but the reduction in setting time was so small that it may not have any impact on the economy of the quarg production. The quantities of milk and cost of enzyme used to produce hydrolysed milk and the impairment of sensory qualities of quargs make processes C and D not look encouraging for industrial application.

5.2 FUTURE RESEARCH NEEDS

From the sensory study of the quargs it was clear that process B induced cooked flavor, slight yellow discoloration and a grainy texture. Though according to expert opinion, overall quality of quarg B was acceptable, it was not an ideal product. These defects probably can be masked if it is used in the production of bakery products or in the production of flavored quarg in combination with fruits, jellies, whipped cream, vanilla or fruit flavors. Quarg-B should be used in the production of above products and their sensory acceptability should be evaluated.

Also flavored quarg , which is very popular in Europe, has not found its place on the shelves of Alberta food stores and therefore special market research effort is needed to develop a market for this variety of quarg.

Production of quarg by the ultrafiltration process can reduce the handling of bulk of fluid and increase the equipment production capacity of quarg per day. This process can also increase the yield and hence profitability of the product by retaining all the milk whey proteins into the protein concentrate. Also, whey permeate resulting from the ultrafiltration of milk can be used in the production of flavored drinks. Therefore, it is reasonable to recommend the continuation of research in quarg technology by the ultrafiltration process.

Determination of amino acid content is the first step in protein quality determination. Since lysine is often the limiting amino acid in many food proteins and is sensitive to processing damage, much attention has been paid to this amino acid. The browning reaction is one result of a processing condition which reduces the lysine content of the protein. It involves the cross linking of a reducing sugar and a free amino group of the protein e.g. ε-amino group of lysine. In the preliminary stages of the Maillard browning reaction, an Amadori compound is formed. Upon hydrolysis with 6N HCl during the amino acid analysis, an Amadori amino acid moiety is released, but it is not available to the rat since intestinal hydrolysis conditions are much milder than 6N HCl. Thus, there is clear distinction between potential nutritional value and actual availability. Accurate information on the digestible amino acids, especially for the essential amino acids is very useful for ranking food proteins.

Amino acid analysis indicated the potential quality of the products in this study. Detailed study is desirable on the estimation of the available lysine and amino acid digestibility for further elucidation of the nutritional quality of quargs.

Quarg, a very versatile product, is copiously used in number of food preparations in Europe. Higher nutritional quality of quarg might lure the North American customer to

consider it in comparison to nutritionally established foods such as an yoghurt in years to come.

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Appendix 1.1 PRELIMINARY SENSORY TESTING OF QUARG

NAME_____ **DATE**_____
Please evaluate the ___, ___, ___, ____, of the quarg in the sequential order

Sensory attribute	— — —			— — —			— — —			— — —		
	1	2	3	1	2	3	1	2	3	1	2	3
Yellow color												
Cooked flavor												
Unclean flavor												
Sweet												
Grainy												

1 = None, 2 = Slight 3 = Pronounced

Appendix 1.2 SENSORY TESTING OF QUARG

NAME_____ DATE_____

PLEASE EVALUATE THE FOLLOWING SENSORY CHARACTERISTICS OF QUARG

(1) COOKED FLAVOR	111	216	447	555
No cooked flavor	___	___	___	___
Trace of cooked flavor	___	___	___	___
Slightly cooked flavor	___	___	___	___
Cooked flavor	___	___	___	___
Very cooked flavor	___	___	___	___
Extremely cooked flavor	___	___	___	___

Comments :

(2) UNCLEAN FLAVOR

No unclean flavor	___	___	___	___
Trace of unclean flavor	___	___	___	___
Slightly unclean flavor	___	___	___	___
Unclean flavor	___	___	___	___
Very unclean flavor	___	___	___	___
Extremely unclean flavor	___	___	___	___

Comments :

Appendix 1.2

(3) SWEETNESS

Not sweet	—	—	—	—
Trace of sweetness	—	—	—	—
Slightly sweet	—	—	—	—
Sweet	—	—	—	—
Very sweet	—	—	—	—
Extremely sweet	—	—	—	—

Comments :

(4) COLOR

Not yellow	—	—	—	—
Trace of yellowness	—	—	—	—
slightly yellow	—	—	—	—
Yellow	—	—	—	—
Very yellow	—	—	—	—
Extremely yellow	—	—	—	—

Comments :

Appendix 1.2

(5) TEXTURE

Not grainy	—	—	—	—
Trace of graininess	—	—	—	—
Slightly grainy	—	—	—	—
Grainy	—	—	—	—
Very grainy	—	—	—	—
Extremely grainy	—	—	—	—

Comments :

Appendix 1.3 Weight of rats on the first day of assay for the PER experiments (g)

Quarg A	Quarg B	Quarg C	Quarg D
54.8	60.8	60.3	55.8
58.4	63.0	64.6	61.5
68.8	54.2	57.2	62.6
59.5	67.1	68.9	65.0
68.4	62.3	59.9	61.2
66.9	65.1	64.6	62.2
67.5	66.9	65.6	65.8
60.8	64.4	64.6	63.1
63.9	65.4	64.8	66.7
70.2	66.3	66.2	66.4
Mean = 63.9	63.6	63.7	63.0

Appendix 1.4 Weight of rats on the first day of assay for the NPU experiments (g)

Quarg A	Quarg B	Quarg C	Quarg D
55.9	63.7	56.6	54.5
62.0	64.4	59.8	61.1
61.9	65.0	63.7	62.9
64.0	60.5	64.8	64.5
60.2	59.4	61.8	64.7
63.4	64.2	69.6	63.9
68.9	64.1	65.3	65.3
61.3	65.1	62.0	63.9
65.8	63.2	60.7	62.6
68.5	64.4	64.2	62.5
Mean = 63.2	63.4	62.9	62.6

Appendix 1.5 Weight of rats on the first day of the assay for the casein and protein free diets (g)

Casein diet	Protein free diet
61.3	64.6
67.1	60.8
42.7	67.1
68.4	71.6
62.3	66.3
69.3	53.5
63.4	62.9
67.4	58.8
64.6	64.5
62.8	58.6
Mean = 62.9	62.9

Appendix 1.6 Raw data for the metabolic faecal nitrogen

Replicate	MFN (g)
1	0.00895
2	0.00819
3	0.00834
4	0.00925
5	0.00864
6	0.00652
7	0.00910
8	0.00880
9	0.00652
10	0.00925

Appendix 2.1 % Total solids (T.S.), % protein content of the whey and the fermentation time (h) for the experimental quargs

Process	1st Replicate	2nd Replicate	3rd Replicate	4th Replicate
T.S. A	60.56	62.20	60.30	63.18
T.S. B	68.20	66.38	66.90	67.52
T.S. C	60.20	56.21	57.90	55.02
T.S. D	62.96	66.27	64.52	66.05
PROT.* A	0.79	0.75	0.82	0.81
PROT. B	0.45	0.48	0.44	0.50
PROT. C	0.73	0.79	0.72	0.76
PROT. D	0.40	0.47	0.45	0.41
F.T.** A	8.08	8.17	8.22	8.25
F.T. B	7.92	8.03	8.07	7.93
F.T. C	7.63	7.50	7.57	7.70
F.T. D	7.58	7.72	7.48	7.70

* = % Protein content of Whey, ** = Fermentation time

Appendix 2.2 Raw data obtained from 8 judges for the sensory quality of quarg A

Judge	Unclean flavor	Sweetness	Yellow discoloration	Cooked flavor	Grainy texture
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	1	1	1
4	1	1	1	1	1
5	1	1	1	2	1
6	1	1	1	1	1
7	1	1	1	2	1
8	1	1	1	1	1

Appendix 2.3 Raw data obtained from 8 judges for the sensory quality of quarg B

Judge	Unclean flavor	Sweetness	Yellow discoloration	Cooked flavor	Grainy texture
1	1	1	4	4	3
2	1	1	3	4	3
3	1	1	3	3	4
4	1	1	2	5	3
5	1	1	4	4	3
6	1	1	3	4	2
7	1	1	3	3	4
8	1	1	3	4	3

Appendix 2.4 Raw data obtained from 8 judges for the sensory quality of quarg C

Judge	Unclean flavor	Sweetness discoloration	Yellow flavor	Cooked texture	Grainy
1	4	3	3	2	1
2	3	3	3	2	1
3	3	4	3	2	1
4	4	4	2	1	1
5	4	3	2	3	1
6	4	4	2	2	1
7	3	4	1	2	1
8	4	4	4	1	1

Appendix 2.5 Raw data obtained from 8 judges for the sensory quality of quarg D

Judge	Unclean flavor	Sweetness	Yellow discoloration	Cooked flavor	Grainy texture
1	5	5	4	4	3
2	4	4	4	5	4
3	4	3	3	5	4
4	3	3	5	4	4
5	4	3	5	3	3
6	4	4	4	5	3
7	3	4	4	4	3
8	4	4	4	4	3

Appendix 2.6 Raw true digestibility data for the experimental quargs and the reference casein

Rep.	Quantity of nitrogen consumed (g)				
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein
1	1.06	1.05	0.85	0.87	0.79
2	1.00	0.99	0.91	0.62	1.00
3	0.98	0.90	0.80	0.90	0.49
4	0.95	0.92	0.90	1.09	1.06
5	0.95	0.93	0.86	1.03	0.98
6	1.00	0.88	1.10	0.95	1.20
7	1.05	1.20	0.94	0.95	1.00
8	1.00	0.93	0.96	1.08	1.02
9	0.98	0.95	0.81	0.89	1.16
10	1.03	1.03	0.93	0.92	0.95

Appendix 2.7 Raw true digestibility data for the experimental quargs and the reference casein

Rep.	Nitrogen in faeces (g)				
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein
1	0.061	0.087	0.069	0.062	0.059
2	0.072	0.075	0.082	0.062	0.057
3	0.097	0.062	0.073	0.079	0.061
4	0.072	0.086	0.073	0.088	0.067
5	0.069	0.062	0.065	0.096	0.086
6	0.085	0.062	0.083	0.078	0.064
7	0.103	0.088	0.070	0.064	0.057
8	0.076	0.080	0.082	0.076	0.054
9	0.081	0.081	0.077	0.069	0.089
10	0.084	0.081	0.073	0.061	0.086

Appendix 2.8 Raw PER data for the experimental quargs and the reference casein

Rep.	Weight Gain (g)				
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein
1	117.05	125.85	122.01	104.88	113.54
2	110.51	121.64	109.81	58.16	94.43
3	121.64	101.83	93.82	130.19	112.56
4	103.71	116.41	115.79	109.84	92.54
5	122.80	81.63	112.61	105.56	100.11
6	122.81	192.37	114.08	101.41	134.87
7	174.05	196.94	136.56	124.25	146.44
8	135.98	132.95	126.84	113.24	123.26
9	136.91	159.44	121.69	143.74	133.71
10	133.01	157.15	85.54	170.85	149.57

Appendix 2.9 Raw PER data for the experimental quargs and the reference casein

Rep.	Quantity of protein consumed (g)				
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein
1	38.97	40.76	38.87	37.30	37.54
2	39.37	40.51	35.42	29.14	29.88
3	42.01	32.68	29.69	43.69	37.19
4	34.34	39.49	39.39	40.78	34.18
5	43.49	25.59	40.40	39.38	31.58
6	39.68	60.68	36.10	36.53	41.13
7	52.48	56.31	39.59	40.30	45.76
8	44.44	40.62	38.00	41.33	37.20
9	43.47	47.45	38.69	47.23	40.56
10	40.81	47.29	28.66	62.58	45.33

Appendix 2.10 Raw NPU data for the experimental quargs,the reference casein and the protein free diet

Rep. Group	Body nitrogen (g)					
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein	P.F.*
1	2.66	2.72	2.26	2.30	2.34	1.395
2	2.55	2.67	2.57	2.29	2.56	1.312
3	2.54	2.39	2.58	2.48	1.97	1.450
4	2.86	2.60	2.65	2.87	2.90	1.562
5	2.60	2.43	2.46	2.89	2.06	1.377
6	2.91	2.67	2.90	2.79	3.12	1.000
7	3.06	3.08	2.63	2.01	2.53	1.397
8	2.87	2.91	2.93	2.90	2.66	1.304
9	2.74	2.74	2.68	2.24	2.90	1.331
10	2.96	2.90	2.69	2.12	2.42	1.215

* P.F. = protein free

Appendix 2.11 Raw NPU data for the experimental quargs, the reference casein and the protein free diet

Rep.	Quantity of nitrogen consumed (g)					
	Quarg A	Quarg B	Quarg C	Quarg D	Ref. Casein	P.F.* Group
1	2.12	2.17	1.41	1.78	1.57	0.0695
2	1.90	2.06	2.03	1.57	1.91	0.0582
3	2.04	1.75	1.85	1.89	1.01	0.0554
4	2.27	2.00	1.90	2.30	2.23	0.0592
5	1.99	1.99	1.77	2.27	1.13	0.0570
6	2.19	2.08	2.24	2.45	2.54	0.0502
7	2.30	2.65	1.94	1.25	1.83	0.0805
8	2.19	2.06	2.44	2.84	2.03	0.0603
9	2.10	2.08	1.79	1.61	2.35	0.0498
10	2.21	2.23	1.92	1.46	1.65	0.0601

* P.F. = Protein free

Appendix 2.12 Amino acid content of the experimental quargs (first replicate)

	g/ 16g Nitrogen			
Amino acid	Quarg A	Quarg B	Quarg C	Quarg D
Arginine	3.586	3.548	3.611	3.643
Histidine	2.844	2.966	2.820	2.847
Isoleucine	5.162	4.779	4.872	4.914
Leucine	9.159	9.368	9.119	9.493
Lysine	7.711	7.914	7.405	7.421
Methionine	2.789	2.516	2.650	2.642
Phenylalanine	5.509	5.191	5.346	5.327
Threonine	3.647	3.790	3.795	3.822
Tyrosine	5.486	5.371	5.511	5.579
Valine	6.771	6.159	6.719	6.811
Alanine	3.299	3.387	3.368	3.485
Aspartic	6.225	6.668	6.510	6.784
Glutamic	18.054	18.108	18.033	18.226
Glycine	1.801	1.765	1.788	1.816
Proline	6.841	6.197	6.745	6.446
Serine	4.911	4.761	4.820	4.907
Cysteine	0.520	0.596	0.613	0.648

The amino acid tryptophan is not included in this analysis.

Appendix 2.13 Amino acid content of the experimental quargs (second replicate)

Amino acid	g/ 16g Nitrogen			
	Quarg A	Quarg B	Quarg C	Quarg D
Arginine	3.668	3.497	3.617	3.664
Histidine	2.903	2.988	2.811	3.003
Isoleucine	4.997	4.850	4.909	4.909
Leucine	9.124	9.493	9.158	9.629
Lysine	7.800	7.984	7.447	7.549
Methionine	2.693	2.574	2.652	2.559
Phenylalanine	5.377	5.204	5.327	5.285
Threonine	3.787	3.933	3.777	3.735
Tyrosine	5.436	5.364	5.594	5.432
Valine	6.683	6.305	6.787	6.350
Alanine	3.343	3.324	3.439	3.401
Aspartic	6.330	6.687	6.573	6.766
Glutamic	17.945	18.118	18.044	18.636
Glycine	1.766	1.782	1.791	1.824
Proline	6.812	6.243	6.735	6.262
Serine	4.830	4.776	4.856	4.972
Cysteine	0.512	0.635	0.639	0.580

The amino acid tryptophan is not included in this analysis.